Contribution of Intramuscular Connective Tissue to the Viscoelastic Properties of Post-Rigor Bovine Muscle

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ABSTRACT

An indepth biomechanical study was performed to characterize more fully the viscoelastic behavior of post-rigor bovine muscle, using samples oriented parallel and perpendicular to the muscle fiber direction. Two enzymes were used to degrade the intramuscular connective tissue (IMCT) to evaluate its mechanical contribution. An unusual stress-strain curve was found in the parallel oriented samples, with enzymolysis affecting the curve at strains larger than 8%. Reductions in stresses in both orientations due to enzymolysis were 50% with extensibility unchanged. Stress relaxation tests indicated that regardless of orientation or treatment the relative stress-relaxation was unchanged. IMCT contributed substantially to the mechanical response only at large deformations.

INTRODUCTION

MECHANICAL EVALUATION of meat has long been a part of determining its textural properties. Studies in this area have attempted to correlate mechanical properties and sensory panel analysis, with generally inconsistent results (Stanley et al., 1971; Szczesniak, 1977; Voisey, 1976). The poor correlation obtained has been stated to be due to the use of a Warner-Bratzler or similar instrument for the mechanical evaluation (Voisey, 1976; Stanley et al., 1971). The device punctures the sample by a complicated deformation process consisting of tension, compression, and shear in different parts of the sample. Only tissue directly under the blade receives the full stress which decreases radially; most of the work required is used in expelling fluid. This apparently simple mechanical test is, therefore, quite complicated. Szczensiak (1977) and Voisey (1976) both stated that because of the above problems there is a need for a more extensive and basic approach in evaluating the mechanical properties of post-rigor muscle.

Work on the mechanical evaluation of meat tissue up to the present has dealt in a wide variety of areas. The emphasis has been on the changes in the mechanical properties during rigor (Eino and Stanley, 1973a, b; O'Shea et al., 1974) or with the effects of various cooking regimes (Bouton and Harris, 1972; Bouton et al., 1972; Locker and Carse, 1976). Work on the contribution of individual components is limited. Hendrickson et al. (1974) performed a study on single muscle fibers and dealt primarily with the effects of hot and cold excision. He used "shear force" of the single fiber as a mechanical measure but did not attempt to correlate his findings to any kind of taste-texture evaluation.

More fundamental studies have been performed to determine the relative contributions of the individual components, and the intramuscular connective tissue (IMCT) has been found to play a major role. Light et al. (1985) studied the texture properties of six bovine muscles and found that the collagenous component of macroscopic muscle connective tissue had the major influence on textural differences, while the endomysium acted on a more subtle level. This work confirmed similar conclusions by Bailey et al. (1979). A study by Mohr and Bendall (1969) found that extracted IMCT from the ox sternomandibularis muscle was more highly cross-linked than the terminal tendon attached to it, which may partially explain the major role of IMCT in the overall mechanical properties. In a related study by Kovaneen et al. (1984) the passive mechanical properties of a fast-twitch and a slow-twitch muscle were examined and related to the relative amounts of collagen. Results clearly indicated a greater load bearing capability of the slow-twitch muscle, which contained more insoluble collagen. Further, when the mechanical data were normalized to the insoluble collagen content of each muscle, no difference was found in the mechanical response, confirming a predominant role of collagen in the mechanical properties. The above work implies a predominant role of IMCT in the mechanical response and hence on the taste-texture properties as well.

Conversely, Magid and Law (1985) found that the stress-strain curve of a skinned muscle fiber, which had no connective tissue, was the same as that of the whole frog skeletal muscle from which it was taken (provided that the increase in sarcomere length was used as the measure of strain). They found further that the rates of stress relaxation in whole muscle and their dependence on stress are similar to those in skinned fibers. They believed that the connective tissue became important at sarcomere lengths greater than 3.8 micrometers (about 80% strain). This result indicated that in a fresh muscle over the range of sarcomere lengths in natural movement, the connective tissue may transmit the force among the fibers, but the elasticity in tension is primarily that of the muscle fibers. However, this result must be viewed from the perspective that it was derived from a live muscle. The effects of rigor should certainly have a significant effect on the mechanical properties, thereby limiting the comparison with post-rigor data.

The above discrepancies clearly indicate the complex nature of both the relative contribution and amount of interaction of the components of muscle to the total mechanical response. The study of intact tissue is, therefore, critical to the complete characterization of its mechanical properties. Related work by Bouton et al. (1975) and Carroll et al. (1978) has shown that post-rigor muscle acts much like a composite material; the myofibrillar proteins are responsible for the initial resistance to deformation, followed by a growing resistance by the IMCT. Carroll et al. (1978) also found that the perimysium was the last tissue visible at rupture, and both found that failure may have occurred at the endo-perimysial junction. Carroll et al. (1978) further found that the muscle fibers were always left intact after failure. This implies that the myofibrillar and other non-IMCT components act both as direct load-bearing components and as a compliant matrix. This and the above cited works clearly indicate the importance of IMCT in the mechanical response of muscle tissue, and the need to study it as an intact composite material unchanged by cooking or mechanical treatments during rigor. Yet, no work known by the authors has examined the fundamental mechanical properties of post-rigor muscle in this way.

In the present study a basic biomechanical approach was used to characterize the response of post-rigor muscle and the relative contributions of its components. This was accomplished by characterizing the stress-strain and the stress-relaxation properties of samples cut parallel or perpendicular to the

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direction of the muscle fibers. Enzymes were also used to chemically degrade a component and to evaluate its contribution to the mechanical response. Enzymes have been used extensively in related studies (Coulson, 1971; Eino and Stanley, 1973b; Hoffmann et al., 1973; Weiss, 1984) as a means to degrade tissue components in mechanical and histological studies. Because collagen is the main tensile component in IMCT and a highly specific enzyme exists for it, the contribution of collagen was investigated in this study. Furthermore, because glycosaminoglycans have been known to contribute to the mechanical response of collagen (Haut and DeCou, 1984), a purified α-amylase was also used to evaluate the contribution of the interfibrillar connective tissue matrix on the mechanical properties. Two novel features of this study were a biomechanical study of the effect of muscle fiber orientation with an emphasis on the contribution of collagen and the use of pseudolinear viscoelastic theory (Fung, 1981) to analyze the stress relaxation response.

MATERIALS & METHODS

Sample preparation

To prepare geometrically regular specimens, post-rigor bovine beef transverse abdominous muscle was obtained as commercially prepared flank steak from a local processor (aged approximately 2 wk), where on-site slaughtering and aging were done. The steaks were cut at 4°C into 2cm x 3cm x 12cm slabs oriented parallel or perpendicular to the muscle fibers. The slabs were frozen with dry ice, then put into an air tight container and stored at −25°C. Within 1 wk 1.0cm x 0.5cm x 9.0cm samples were cut with a die. Only those samples that did not contain any visible blood vessels or large fat-connective tissue structures were used. Samples used for fresh tissue tests were so prepared, allowed to stand for 20 min to bring them up to room temperature (22°C) and then tested.

All other samples were lyophilized and vacuum-rehydrated with incubation buffer. This was done to bypass the significant difficulties encountered with enzyme diffusion in the large samples required for mechanical testing. Vacuum-rehydration was performed by evacuating the samples to 10 torr for 10 min. Room temperature (22°C) incubation buffer was introduced until the samples were completely covered; then they were returned to atmospheric pressure. The incubation buffer consisted of 150 mM NaCl, 50 mM Tris-HCl, 5 mM $CaCl_2$, 0.5% NaN_3 as a bactericide, and enzyme for the treated samples. The buffer was allowed to fully permeate the samples during storage at 2°C for 48 hr with gentle agitation. After this period the pH was readjusted to 7.4 for collagenase and 6.9 for amylase. Immediately afterward, the collagenase treated samples were incubated for 2 hr at 37°C, and the amylase treated samples were incubated for 5 hr at 25°C. The controls of both enzyme treatments were prepared identically except with no enzyme. The enzymes used were a purified collagenase from *Cl. histolyticum* (Sigma Chemical Co., St. Louis, MO, Cat. No. C-0773) and a porcine pancreatic alpha-amylase (Sigma, Cat. No. A-4268, treated with dimethylsulfonyl fluoride). Concentrations used were 60 units/mL buffer for collagenase or 413 units/mL buffer for amylase (units as defined by Sigma). These concentrations and incubation periods were found to be the lowest saturating values that produced an effect equivalent to higher concentrations.

Mechanical testing

Stress-strain behavior studies were carried out in tension with an Instron Universal Tester equipped with flat-plate plastic clamps with a gripping surface of 2.5cm x 3.0cm and with 0.1cm deep grooves ruled every 0.2cm. The stress transducer was a BH 50 Newton (N) load cell which was mounted above the upper grip, excited and amplified by a Sensotec Model SA-BII. Data acquisition was performed digitally by a Keithley Series 500 data acquisition system controlled by an IBM-PC microcomputer, with data sampled at 60 Hz. Mechanical testing began by removing the sample from the incubation buffer and mounting it onto the Instron. The sample was slowly extended until a preload of approximately 0.04N (0.01N for the perpendicular samples) was initially achieved and the extension halted. The length of the sample at this point was taken to be the initial length (typically 2 cm). The sample was then extended at a constant rate of 12.7 cm/min (corresponding to a strain rate of 10%/sec to 15%/sec) until complete rupture occurred. Only those tests in which failure occurred near the center (no grip failures) were used. Immersion was not required because of the short time required for testing. The load reading after rupture was close to zero (+/− 0.05N), indicating that the above protocol gave a good approximation to the actual start of the stress-strain curve.

Stress relaxation was studied in an apparatus with solenoid-activated levers to stretch six samples simultaneously in about 70 milliseconds (ms). The grips, stress transducers, and data acquisition system were identical to those used in the stress-strain behavior studies, the Keithley unit here being used as a multiplexer to acquire data from six channels simultaneously. For each channel, data were sampled at 500 Hz for the first 3 sec, then at the same rate in 200-point sampling arrays taken once per minute. The mean of each array was taken as the value of the load at the time of the array; this method was utilized to reduce signal noise. The initial three second data were used to extrapolate the load to zero time (or initial load) since mechanical vibration of the testing apparatus immediately following extension prohibited direct measurement of this parameter. A test began with the samples prepared identically as above. Each sample was mounted vertically between a stationary and a moving clamp with ample slack. The sample was then immersed in a testing buffer at room temperature (22°C), consisting of 150 mM NaCl and 20 mM sodium phosphate set at pH 7.4 for collagenase and 6.9 for amylase. The initial length was found as in the stress-strain tests, and the samples were extended by 30% of that length and held at this strain level and temperature for 20 hr. This long test time was chosen to fully characterize the stress-relaxation behavior. Any deleterious effects of long-term immersion were thought to be small and bacteriological effects minimal due to the NaN_3 infused in the sample. A strain level of 30% was chosen from the stress-strain curves as a value at which the IMCT were significantly extended but still well below yield. After the test was completed, each sample was removed and frozen at −25°C in a tightly sealed container for later examination.

Mechanical parameters

All load values were converted to stress by using the initial cross-sectional area of 0.5 cm^2. Strains are given as extension/initial length and expressed as percent. The maximum tangent modulus of the stress-strain curve was calculated from an iterative 19-point "window" sweeping up the curve in which the best straight line was calculated for each window. The maximum tangent modulus was taken as the maximum value encountered, with the stress and strain values at this point taken from the endpoint of this window.

Fung (1981) has shown that the stress-relaxation response of tissue can be separated into time-dependent and strain-dependent factors:

$$S(e,t) = E(e)T(t) \qquad (1)$$
$$T(0) = 1$$

where S is the stress on the tissue, E is a function only of the strain (e), and T is a function only of the time (t). In a stress-relaxation test T(t) is then the reduced stress $[S_t]$, so that

$$T(t) = [S_t] = S(e,t)/S(e,0)$$

where S(e,t) is the stress at a strain level e at time t, and S(e,0) the initial stress at zero time at that strain level. In the current study, the T(t) was found by fitting the reduced stress relaxation curves to the empirical equation:

$$[S_t] = A(t+1)^B \qquad (2)$$

This equation was found to fit the data better than the more common logarithmic fit used for most tissues (Fung, 1981). With the use of Eq. (2) the exponent B becomes a measure of the stress-relaxation relative to the initial stress.

All quantitative effects were tested for significance by means of a Student's t-test at a 95% level of confidence [$p < 0.05$], with a pooled estimate of the standard error.

RESULTS

Stress-strain behavior

Fresh tissue. Figure 1 is a plot of a typical stress-strain curve for parallel tissue; it also depicts the mechanical parameters studied. Table 1 lists the data derived from these tests. Here, an unusual biphasic response up to the S^m can be seen. In the initial region (labeled R1), which ended at about 13%

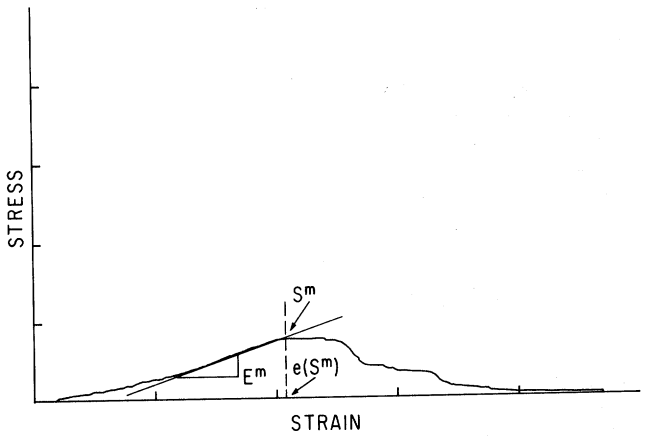

Fig. 2—A typical stress-strain curve (arbitrary units) for perpendicular oriented fresh tissue. Note that it is scaled the same as Fig. 1 for comparison. Definitions of the other parameters can be found in the Nomenclature.

Table 2—Stress-relaxation test results for collagenase treated samples incubated at 37°C for 2 hr

	Parallel		Perpendicular	
Parameter	Control	Treated	Control	Treated
S_0 (KPa)				
mean	37.08	22.96	2.20	1.10
s.e.	12.86	8.42	0.66	0.22
n	11	12	5	3
S_1 (KPa)				
mean	17.68	10.56	1.10	0.38
s.e.	5.20	3.38	0.30	0.20
n	11	12	5	5
S_{20} (KPa)				
mean	3.14	1.76	0.22	0.18
s.e.	0.84	0.72	0.06	0.12
n	11	12	5	5
$[S_1]$				
mean	0.488	0.469	0.522	0.344
s.e.	0.054	0.044	0.134	0.129
n	11	12	5	3
$[S_{20}]$				
mean	0.092	0.078	0.105	0.140
s.e.	0.030	0.020	0.033	0.076
n	11	12	5	3
B				
mean	−0.2608	−0.3021	−0.3063	N/A
s.e.	0.0307	0.0307	0.0203	
n	11	12	5	

samples required a larger deformation to induce failure; but once induced, failure occurred more rapidly than in the controls.

Stress relaxation

Orientation effects. Results for the stress-relaxation tests for control and collagenase treated samples are listed in Table 2. Differences between orientations were greater then those found for the stress-strain behavior tests. All stresses (S_0, S_1, S_{20}) in the perpendicular samples were only 6% ($P \leq 0.05$) of the parallel. This was in contrast to the difference of 26% ($P \leq 0.05$) found for the S^m in the stress-strain tests. The disparity between orientation effects on the stress values between the two types of tests was not unexpected since the tests are quite different in nature (progressive deformation to failure as compared to the response to an instantaneous increase in deformation). However, there was a possibility that some damage to the perpendicular samples might have occurred in the stress-relaxation tests due to the extremely high strain rate used here (400%/sec compared to 10%sec-15%/sec) since the perpendicular samples were more fragile than the parallel ones. This fragility would cause the decrease in the relative difference in stresses found between the orientations here. However, in visual inspections of the perpendicular samples after testing they appeared to have maintained mechanical integrity. The reduced stresses [S_1] showed no differences between orientations, indicating that similar relaxation mechanisms exist between the two orientations. The decay parameter B did show 17% ($P \leq 0.05$) increase in value for the perpendicular tissue. However, when the mean fitted curves (Eq. 2) for the parallel and perpendicular tests were plotted together, this difference was seen to be negligible. Overall, the results showed that at 30% strain the stress-relaxation process was essentially the same regardless of the muscle fiber orientation.

Collegenase effect. In the parallel samples treated with collagenase the S_0 was reduced to 62% ($P \leq 0.05$) of the control value, which was almost identical to that found for the S_m in the stress-strain behavior tests. The S_1 and S_{20} indicated a small but continual increase in this difference throughout the test. This increase was supported by a 16% decrease ($P \leq 0.05$) in B (more negative) with collagenase treatment. However, this difference was seen to be quite small when the mean fitted stress-relaxation curves (Eq. 2) were plotted together. Thus, although the encountered stresses were considerably lessened, the relative stress-relaxation behavior was essentially the same after collagenase treatment.

For the perpendicular samples S_0 was reduced to about 50% ($P \leq (0.05)$ of the control value, again like that found for the S^m in the stress-strain behavior tests. However, after the initial extension, the relaxation behavior changed substantially in the treated samples. The [S_1] for the treated samples was only 0.34, as opposed to 0.54 for the controls. Past 1 min, stress-relaxation slowed considerably in the treated samples but continued significantly in the controls ($[S_{20}] = 0.105$, $B = -0.3063$). Due to the considerably reduced relaxation, the power equation [Eq. (2)] did not fit the perpendicular data well ($r^2 = 0.366$), so the value for B was not a valid measure of the stress-relaxation here. Closer examination of the stress-relaxation curves showed that stress-relaxation ceased in the treated samples past 1 hr, after which the stress remained constant. Overall, the encountered stresses were reduced as in the stress-strain behavior tests, and stress-relaxation was also considerably reduced by collagenase treatment. Because of the above difficulties and time constraints on the study, it was decided not to use the perpendicular samples in the amylase tests.

Collagenase and amylase. Results for these tests are listed in Table 3. The different incubation time and temperature used in these tests did not alter the proportional decrease in S_0 for the samples treated with collagenase alone. The change in B from the value of the prior tests was not thought to be due to any change in the ability of collagenase to degrade the native collagen, since the proportional reduction of S_0 was equivalent. Rather it was thought to be due to a greater amount of cytoplasmic and other native material exuded from the sample during the longer incubation time used here. Preliminary experiments clearly indicated the longer a lyophilized sample was immersed in buffer, the more material was exuded, causing a lowering in the stress-bearing capability and stress relaxation in the tissue. Eino and Stanley (1973a) found a similar decrease in the breaking strength of fresh tissue with continued immersion for up to 3 hr. Therefore, direct comparison of the long-term stress-relaxation properties between the two separate incubation protocols (i.e., Tables 2 and 3) is not possible. However, it is evident from the equal proportional reduction of S_0 in both treatments that the collagen was affected equally.

Amylase alone reduced S_0 by 43% ($P \leq 0.05$) of the control, very close to the effect of collagenase. The B showed a small

Table 3—Stress-relaxation test results for Amylase, collagenase, and combination samples

S_0 (KPa)				
mean	20.16	11.80	11.38	8.840
s.e.	5.08	3.06	1.98	2.10
n	6	4	9	5
S_1 (KPa)				
mean	9.18	6.30	6.62	5.10
s.e.	1.64	1.76	1.02	1.46
n	6	4	9	5
S_{20} (KPa)				
mean	1.60	1.40	1.68	1.34
s.e.	0.12	0.60	0.38	0.44
n	6	4	9	5
$[S_1]$				
mean	0.463	0.533	0.588	0.569
s.e.	0.041	0.044	0.078	0.055
n	6	4	9	5
$[S_{20}]$				
mean	0.084	0.116	0.150	0.150
s.e.	0.021	0.026	0.032	0.017
n	6	4	9	5
B				
mean	−0.2701	−0.2546	−0.2319	−0.2179
s.e.	0.0161	0.0314	0.0369	0.0145
n	6	4	9	5

($P \leq 0.05$) decrease from the control but was not different from the collagenase.

When both enzymes were used together, the S_0 was reduced by 56% ($P \leq 0.05$), about 14% greater ($P \leq 0.05$) than either enzyme alone. The B was about 20% smaller than the control (but $P \geq 0.05$), indicating a similar decay to either enzyme alone.

DISCUSSION

THE STRESS-STRAIN CURVE for the parallel tissue reported here (Fig. 1) is unlike most reported for muscle (live or post-rigor). A similar response was found only for the deep pectoralis muscle (Bouton et al., 1975). The initial deformation region, which we call R1, has an appearance similar to fresh muscle, but the latter extends to well over 100% strain, even if measured by sarcomere lengths (Magid and Law, 1985) instead of sample length. After this, the curve showed a linear yielding region starting at about 13% strain. This type of response may be characteristic of post-rigor muscles that have a high degree of muscle fiber orientation. Our results are consistent with previous assignment of the main stress bearing components in R1 to the muscle fibers (Magid and Law, 1985). This assignment is supported by the large reduction of E^m when the muscle fibers are perpendicular (assuming they have no load bearing capability in this configuration), and to the total lack of effect of collagenase in R1.

Collagen appeared to contribute significantly to the stress-strain properties of region R2. Collagenase reduced the E^y, S^m, and $W(S^m)$ substantially, all of which were measures of the stress bearing capability of the tissue. If it is accepted that the stress transmitted through the perpendicularly oriented tissue is supported by the connective tissue alone, one can understand why the E^m in these samples is comparable to the E^y in the parallel samples (assuming that all the muscle fibers in R2 are completely ruptured and the connective tissue is the only load bearing component). This assumption is further supported by the similar reductions in value induced by collagenase for the perpendicular E^m (49%) and the E^y (57%).

Yet collagenase does not completely reduce to zero the stress bearing capability of the connective tissue. Furthermore, the $e(S^m)$, e^r, and the ratio $W(S^m)/W^r$ were essentially unchanged by the enzyme. This leads to the conclusion that degrading collagen significantly reduces the stress-bearing capability of the tissue but does not alter its extensibility. This has implications on the taste-texture of meat; the collagenase-proteolyzed meat would be more pliant since its resistance to deformation would be reduced.

Stress-relaxation results showed that the reduced stresses [S_t] were essentially equal between the two orientations, although the measured stresses were much higher in the parallel samples. The degree of stress-relaxation, as determined by the exponent B, was very similar when the muscle fibers were rotated 90°. We concluded that these fibers were not involved in stress-relaxation.

Treatment with collagenase reduced the stresses by an amount almost identical to that found in the stress-strain behavior tests, regardless of tissue orientation. Stress-relaxation was essentially unaffected by collagenase treatment in the parallel samples but was reduced substantially in the perpendicular ones. For these tests, it was possible that some type of rupturing occurred either during the initial extension or during the first hour (or both). However, it is unlikely that any measureable amount of fiber damage other than that caused by collagenase occurred during the initial extension, since the S_0 was reduced by the same amount as in the stress-strain behavior tests. A more plausible explanation is that the perpendicular treated samples were unable to withstand an applied strain for long time periods, and the stress continued to decay until an equilibrium stress occurred that the fibers could withstand. This explanation would agree with our ability to perform successful stress-strain behavior tests on the same tissue. The perpendicular samples were in general more fragile than their parallel counterparts, and testing them was difficult. Overall, stress-relaxation of treated tissue could only be measured with certainty in the parallel orientation. The results for these tests imply that the relaxation process does not involve collagen to the degree that we could measure.

Finally, when samples were treated with amylase alone and in combination with collagenase, the treatment produced very similar effects compared to collagenase; both the reduced stresses and stress-relaxation were nearly identical. It appeared that both enzymes attacked the same structural element, thus producing similar mechanical effects. Within the limits of this study, the stress-relaxation behavior, regardless of treatment and orientation, was basically unaffected. While all the structural components of the post-rigor tissue evidently contributed to the time-independent elasticity E(e) in equation (1), neither myofibrils nor connective tissue contributed to the time dependence [T(t)]. Possible contributors are perhaps the intermediate filaments that bind together the contractile filaments within the muscle cells (Loewy et al., 1985) or fluid redistribution within the tissue, both internal and external to the connective tissue matrix.

The methodologies used in this study were clearly successful in their abilities to evaluate the complex mechanical properties of post-rigor muscle. This should be of benefit not only in determining basic properties of post-rigor muscle but to those interested in meat-texture evaluation. Improved knowledge of how individual components contribute to the total response and their mutual interaction may lead to important developments in meat-texture research.

NOMENCLATURE

Stress strain behavior*

Parameter	Units	Definition
E^m	KPa	Maximum tangent modulus of the stress-strain curve.
$S(E^m)$	KPa	Stress at E^m.

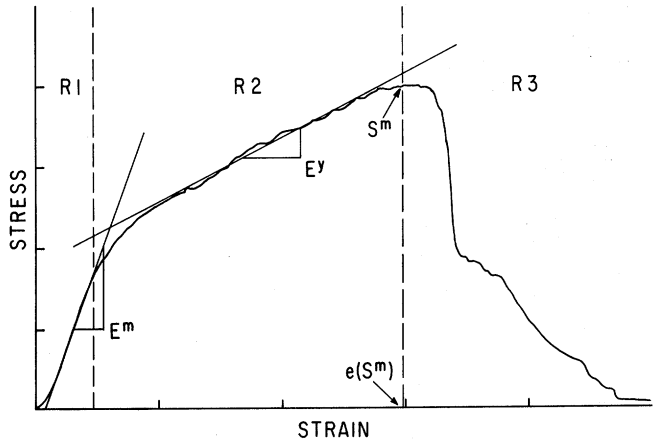

Fig. 1—A typical stress-strain curve (arbitrary units) for parallel oriented fresh tissue. R1 is defined as region 1, R2 as Region 2, and R3 as region 3. Definitions of the other parameters can be found in the Nomenclature.

strain, the curve is quadratic. The end of this region, where the E^m occurs, is followed by an extensive yielding region (labeled R2) which was substantially longer than R1. The slope of the linear portion of R2, referred to as E^y, had a typical value 1/7 of E^m, and ranged from 25% to 70% strain. The S^m had a mean value of 121.7 KPa and occurred at about 82% strain, followed by a region (labeled R3) characterized by a rapid return to zero stress which occurred at 176% strain at full rupture. The perimysium was always the last visible component left intact just before total rupture with the muscle fibers completely ruptured, as reported by Carroll et al. (1978).

Results for the perpendicular tissues are also listed in Table 1, and a typical plot of the stress-strain behavior is shown in Fig. 2. Here no biphasic behavior was found. The stress-strain curve was quadratic up to 74% strain; then a gradual yielding occurred up to S^m of 24.1 KPa at 107% strain followed by a slow drop in stress until total rupture at 175% strain.

Orientation effects. The effects of orientation for fresh tissue were drastic (Table 1). Both the perpendicular E^m and W^r were 80% less ($P \leq 0.05$) than the parallel values. The perpendicular S^m was only 37% of the parallel ($P \leq 0.05$). Conversely, the e^r showed no change between orientations, and when the E^y was compared to the perpendicular E^m, their values agreed closely.

Effects of lyophilization. In parallel orientation, lyophilized tissue showed the same biphasic behavior as did the fresh samples (Fig. 1); however, quantitative differences did occur. All measured mechanical parameters in R1 substantially decreased in value ($P \leq 0.05$, Table 1) when compared to the fresh samples. The W^r, $W(S^m)$, and S^m also all decreased ($P \leq 0.05$). The strains at S^m and at rupture both increased by about 20% strain (but $P \geq 0.05$), implying lyophilization may have slightly increased the extensibility of the sample.

The lyophilized samples with perpendicular fibers again showed a smooth quadratic response up to S^m. The E^m was higher by 42% ($P \leq 0.05$), but the S^m and the $S(E^m)$ were unchanged. The strains at E^m and S^m were lowered by half ($P \leq 0.05$), reducing the work at these respective points by 50%, and the e^r was reduced by 19% ($P \leq 0.05$). Clearly, the extensibility of the tissue was significantly reduced by lyophilization. The W^r was reduced by 21% ($P \leq 0.05$), and the $W(S^m)/W^r$ went from 55.8% in the lyophilized controls to 34% ($P \leq 0.05$). The behavior of the W^r and $W(S^m)/W^r$ when considered with the above data implied a substantial change in the mechanism of failure.

Effects of collagenase. For the parallel samples the entire R1 of the stress-strain curve was completely unchanged from that of the lyophilized control. No R1 parameter showed a statistically significant change in value (Table 1). In R2, however, large changes were found. The W^r was reduced by 37% ($P \leq 0.05$), with a similar reduction in the S^m ($P \leq 0.05$). The decrease in S^m is similar to that reported by Eino and Stanley (1973b) of 50% for fresh bovine muscle. The E^y was also reduced by 43% ($P \leq 0.05$) from that of the lyophilized control. Conversely, both e^r and the $W(s^m)/W^r$ were unchanged.

For the perpendicular samples the effects of collagenase were substantially greater (Table 1). The E^m was reduced by 50% ($P \leq 0.05$) and the S^m by 55% ($P \leq 0.05$). The W^r was reduced by 61% ($P \leq 0.05$), 20% more than in the parallel tissue. Remarkably, the parameter $W(S^m)/W^r$ was larger than in the control by 62% ($P \leq 0.05$), which could imply a more gradual rupture process, yet the e^r was unchanged. Evidently, treated

Table 1—Stress-strain behavior results for parallel and perpendicular samples treated with collagenase and incubated for 2 hr @ 37°C

	Parallel			Perpendicular		
		Lyoph.			Lyoph.	
Parameter	Fresh	Control	Treated	Fresh	Control	Treated
E^m (KPa)						
mean	598.4	213.0	241.8	55.0	78.1	37.9
s.e.	141.0	38.0	74.0	18.2	20.1	6.9
n.	5	9	10	7	4	6
$S(E^m)$ (KPa)						
mean	45.82	13.14	13.52	16.84	15.84	6.54
s.e.	14.72	2.72	3.52	9.36	2.96	3.10
n	5	10	10	8	4	6
$e(E^m)$ (%)						
mean	13.1	7.7	7.6	74.2	40.5	45.3
s.e.	4.2	2.2	1.7	22.9	8.9	15.7
n	5	9	10	7	4	6
$W(E^m)$ (Pa)						
mean	21.5	4.2	4.6	35.1	23.3	11.2
s.e.	10.1	1.5	1.3	19.1	5.2	6.9
n	5	9	10	7	4	6
S^m (KPa)						
mean	121.7	92.0	58.3	24.1	23.7	10.6
s.e.	8.2	17.8	10.5	11.5	3.3	3.5
n	5	10	10	8	4	6
$e(S^m)$ (%)						
mean	82.1	101.6	79.0	107.3	56.1	74.4
s.e.	25.3	23.2	15.0	30.2	16.0	15.0
n	5	9	10	7	4	6
$W(S^m)$ (KPa)						
mean	68.9	55.4	29.0	12.0	5.5	3.4
s.e.	2.79	1.6	8.6	8.6	1.4	1.0
n	5	9	10	7	4	6
E^y (KPa)						
mean	84.4	105.0	59.4	*	*	*
s.e.	30.0	35.9	17.7			
n	5	9	10			
e^r (%)						
mean	175.7	197.3	182.3	176.8	143.0	128.2
s.e.	58.8	47.0	64.3	19.8	38.1	51.7
n	5	9	10	7	4	6
W^r (KPa)						
mean	99.1	80.6	48.1	20.6	16.2	6.4
s.e.	34.7	17.2	14.6	12.4	5.3	2.6
n	5	9	9	7	4	6
$W(S^m)/W^r$ (%W^r)						
mean	68.5	68.4	62.2	55.8	34.0	55.0
s.e.	8.2	12.8	11.3	13.9	6.0	11.0
n	5	10	10	8	4	6

* Not applicable

$e(E^m)$	%	Strain at E^m.
$W(E^m)$	KPa	Work done on the sample up to E^m.
S^m	KPa	Maximum stress.
$e(S^m)$	%	Strain at S^m.
$W(S^m)$	KPa	Work done on the sample up to S^m.
E^y	KPa	The tangent modulus at the linear portion of the yield region (parallel orientation only).
e^r	%	Strain at complete rupture.
W^r	KPa	Work done on the sample up to rupture (total work).

Stress Relaxation Behavior

Parameter	Units	Definition
S_0	KPa	The initial stress (extrapolated) of the deformed sample.
S_1	KPa	The stress at one min.
S_{20}	KPa	The stress at 20 hr.
$[S_1]$	-	Reduced stress at 1 min, equal to S_1/S_0.
$[S_{20}]$	-	Reduced stress at 20 hrs, equal to S_{20}/S_0.
B	-	Stress-relaxation decay exponent of Eq. (2).

*All tangent moduli are presented as stress/100% strain, and all work values are in Stress × Strain × 100, both expressed in KPa.

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Ms received 4/29/87; revised 7/2/87; accepted 7/3/87.